# Epidemiologic and Genomic Analysis of SARS–CoV–2 Delta Variant Superspreading Event in Nightclub, the Netherlands, June 2021

Jelle Koopsen,[1] Catharina E. van Ewijk,[1] Roisin Bavalia, Akke Cornelissen, Sylvia M. Bruisten, Floor de Gee, Alvin X. Han, Maarten de Jong, Menno D. de Jong, Marcel Jonges, Norin Khawaja, Fleur M.H.P.A. Koene, Mariken van der Lubben, Iris Mikulic, Sjoerd P.H. Rebers, Colin A. Russell, Janke Schinkel, Anja J.M. Schreijer, Judith A. den Uil, Matthijs R.A. Welkers, Tjalling Leenstra, on behalf of the ARGOS consortium

We report a severe acute respiratory syndrome coronavirus 2 superspreading event in the Netherlands after distancing rules were lifted in nightclubs, despite requiring a negative test or vaccination. This occurrence illustrates the potential for rapid dissemination of variants in largely unvaccinated populations under such conditions. We detected subsequent community transmission of this strain.

Because of decreasing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) incidence rates in the Netherlands at the time, the government of the Netherlands lifted most restrictions on June 26, 2021 (week 25) (1). The mandate to stay at home and get tested if experiencing symptoms remained. However, wearing of facemasks was no longer mandatory if a distance of $\geq$1.5 meters could be maintained. Event attendees who were fully vaccinated or had tested negative for SARS-CoV-2 within the previous 40 hours (testing-for-access) did not have to wear facemasks or maintain 1.5-meter physical distancing. Persons meeting 1 of those criteria (tested or fully vaccinated) were given a QR code in the CoronaCheck application, commissioned by the government of the Netherlands (2), which allowed them access to events.

Author affiliations: Amsterdam University Medical Centres, University of Amsterdam, Amsterdam, the Netherlands (J. Koopsen, A.X. Han, M.D. de Jong, M. Jonges, S.P.H. Rebers, C.A. Russell, J. Schinkel, M.R.A. Welkers); Public Health Service Amsterdam, the Netherlands. (C.E. van Ewijk, R. Bavalia, A. Cornelissen, S.M. Bruisten, F. de Gee, M. de Jong, N. Khawaja, F.M.H.P.A. Koene, M. van der Lubben, I. Mikulic, A.J.M. Schreijer, J.A. den Uil, M.R.A. Welkers, T. Leenstra)

Shortly after June 26, coronavirus disease (COVID-19) cases surged in the greater Amsterdam region of the Netherlands (Appendix 1 Figure 1, https://wwwnc.cdc.gov/EID/article/28/5/21-2019-App1.pdf). Most infections were among young adults 18–30 years of age (Appendix 1 Figure 2), of whom only 14% were fully vaccinated at that time (3). A steep increase in reported clusters related to the hospitality sector, particularly bars and discotheques, was observed in the following weeks; 121 clusters were reported in week 27 compared with an average of 4 clusters/week in weeks 21–25 (Appendix 1 Figure 3). To gain insight into the case surge and transmission dynamics, we investigated an outbreak linked to a nightclub event in central Amsterdam on June 26. We examined whether the high number of cases linked to the nightclub were the result of a superspreading event or the attendance of multiple infectious persons.

## The Study

In the Netherlands, confirmed infections are reported to the local Public Health Service (PHS), and source and contact tracing is performed with a telephone interview. Data are obtained on sociodemographics, date of symptom onset, symptoms, vaccination status (and, if applicable, vaccine type, number of doses, and dates of administration) and locations the index-patient visited during the incubation and contagious periods. Medical ethics clearance for this study was not required (Appendix 1).

We defined a case as illness in a person who visited the nightclub on June 26, tested positive for

---

[1]These authors contributed equally to this article.

SARS-CoV-2 within 14 days, and whose status was reported to the PHS. Cases were identified passively: persons were included only if they indicated during their PHS interview that they had visited the nightclub on June 26. The nightclub has an estimated capacity of 150 persons and was reported to be at full capacity that evening with attendees dancing and singing to loud music. A total of 60 confirmed COVID-19 cases were linked to the nightclub, raising suspicion of a superspreading event. Onset of symptoms occurred during June 27–July 3. Most case-patients were not fully vaccinated (defined as 14 days after completion of the vaccination series [Appendix 1 Figures 4, 5]): 4 (7.4%) persons were fully vaccinated and 41 (76%) were unvaccinated (Table). Most cases were in young adults (mean age 21.1 years [SD 3.3 years]) and women (60%), and most persons reported COVID-19–associated symptoms (93%). In 61% of cases, no other potential source for transmission besides the nightclub event was indicated. Of the 60 confirmed cases, 33 persons lived in the Amsterdam region and 27 resided in other regions (Table).

Samples from 23/60 cases were available for sequencing, of which 3 were not eligible because of high cycle threshold values (>32). For 19/20 samples, we successfully obtained full genome sequences; all belonged to PANGO-lineage B.1.617.2 (4), which was denoted as variant of concern Delta by the World Health Organization (5) (Appendix 1 Table). To provide for phylogenetic context, we included weekly surveillance samples from the Amsterdam region (n = 421) in the analyses, as well as all Delta variant sequences from the Netherlands available in the GISAID database (https://www.gisaid.org; n = 4,465) (Appendix 2 Table, https://wwwnc.cdc.gov/EID/article/28/5/21-2019-App2.pdf) on August 1, 2021. All nightclub-associated genomes showed characteristics of a superspreading event: a tight phylogenetic cluster closely related in time (June 27–July 3) (Appendix 1 Figure 4) and genomic diversity (Figure 1). The pairwise genetic distance between all sequences was ≤2 single-nucleotide polymorphisms (Appendix 1 Figure 6), comparable to previously observed superspreading events (6). In addition, all sequences formed a monophyletic cluster marked by a specific single-nucleotide polymorphism combination: a Delta variant with C4321T in the presence of (wild-type) 22792C. In our dataset, all viruses with this combination collected before July 1 were sampled from persons who were at the nightclub. This combination was not observed in our dataset or in any Netherlands Delta sequences (n = 4465) from the GISAID database before June 26 (Appendix 1 Figure 7). Furthermore,

randomly collected surveillance samples in the region from the weeks preceding the nightclub event showed diverse viruses circulating in the Amsterdam region (Appendix 1 Figure 8), and samples collected from 2 other nightclubs on June 26 also showed different lineages (Appendix 1 Figure 9). This finding makes multiple introductions at the nightclub with a highly prevalent, highly similar variant unlikely. In all, these findings strongly suggest a single introduction of the C4321T + 22792C variant, which was amplified by superspreading at the nightclub.

Since the introduction of C4321T + 22792C, the variant has been increasingly detected in random genomic surveillance from the Amsterdam region: no surveillance samples were detected in week 26 compared with 33% of samples in week 28 (Figure 2). This lineage was introduced the weekend nightclubs were opened and has clearly propagated in the community, where subsequent transmission of the lineage occurred.

## Conclusions

This study illustrates the amplification of a specific linage in a largely unvaccinated group under circumstances such as those observed in a nightclub where

**Table.** Descriptive statistics of 60 persons with confirmed SARS-CoV-2 infection after nightclub event, Amsterdam, the Netherlands, June 2021*

| Characteristics | No. (%) |
|---|---|
| Sex | |
|   F | 34 (60) |
|   M | 23 (40) |
|   Unknown | 3 |
| Mean age, y (SD) | 21.1 (3.3) |
|   Unknown | 3 |
| Symptoms | |
|   Symptomatic | 55 (93) |
|   Asymptomatic | 4 (7) |
|   Unknown | 1 |
| Vaccination status | |
|   Fully vaccinated† | 4 (7.4) |
|   Incomplete vaccination | 9 (17) |
|   No vaccination received | 41 (76) |
|   Unknown | 6 |
| PHS region | |
|   PHS Amsterdam | 33 (57) |
|   Other PHS region | 25 (43) |
|   Unknown | 2 |
| Other self-reported potential sources | |
|   None: only nightclub on June 26th | 35 (61) |
|   Other hospitality sector | 17 (30) |
|   Education | 1 (1.8) |
|   Social gathering | 2 (3.5) |
|   Supermarket | 1 (1.8) |
|   Vaccination location | 1 (1.8) |
|   Unknown | 3 |

*PHS, Public Health Service; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
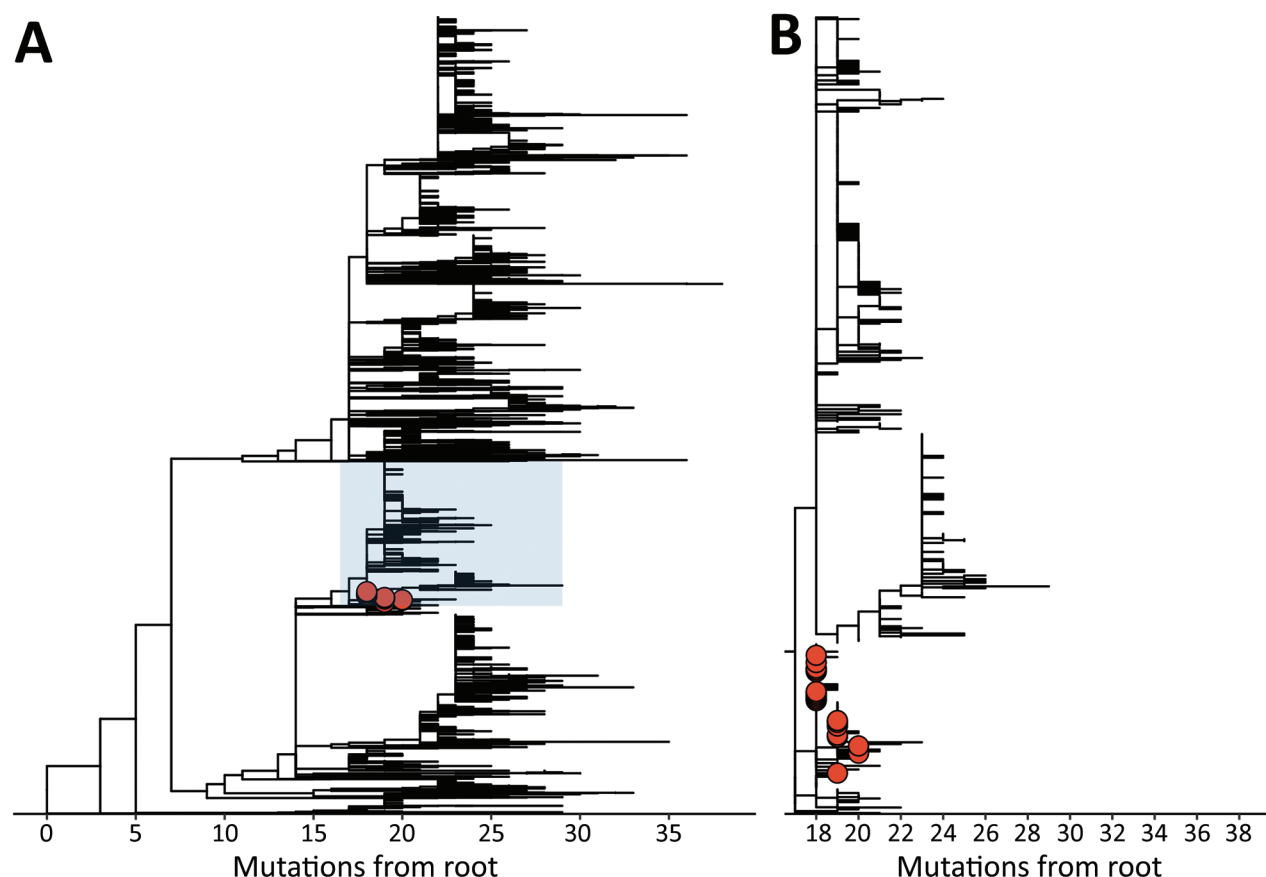†Fully vaccinated defined as 14 days after completion of vaccination series.

**Figure 1.** Clustering of severe acute respiratory syndrome coronavirus 2 cases related to nightclub event, Amsterdam, the Netherlands, June 2021. Red circles indicate sequences linked to the nightclub. A) Maximum-likelihood tree of all sequences (n = 4,905) in the dataset. B) Magnification of the clade (highlighted in blue in panel A) containing sequences linked to the nightclub (n = 1,663). Branches without tips depict other Netherlands Delta variant sequences derived from GISAID (https://www.gisaid.org).

social distancing measures and facemask requirements were lifted, despite a testing-for-access policy. In addition, our results highlight the consequence of superspreading events on subsequent transmission dynamics of SARS-CoV-2 in the community. Investigating an outbreak on June 26, 2021, the first date that social distancing measures were lifted under testing-for-access conditions, enabled us to isolate a single SARS-CoV-2 transmission event.

The role of superspreading in SARS-CoV-2 transmission has been highlighted previously (6,7), also in the context of nightclubs (8,9). Considering the potential of SARS-CoV-2 to be transmitted through aerosols (10,11), nightclubs can be a high-risk setting because of poor ventilation and sustained overcrowding. Our findings suggest that the rapid surge in cases in July 2021 was at least partially driven by superspreading events such as the event we describe.

In particular, testing-for-access, as it was put in place in the weeks following June 26, provided

opportunity for infectious persons to slip through. Access was provided immediately after a single Johnson & Johnson/Janssen vaccination (https://www.janssen.com) (too soon), a negative antigen test result was valid for 40 hours (too long), and checking of QR codes was reported to be inconsistent at some venues (12,13).

This study used data collected for nonresearch purposes during scaled-down source and contact tracing and has limitations. First, cases were passively included, which could underestimate the true extent of the outbreak, because asymptomatic cases or cases tested only by self-administered antigen tests might have been missed. This factor could also explain the high percentage of symptomatic cases (14). Nevertheless, we believe this factor did not result in a biased selection of cases. Second, we conducted genomic analysis for only 1/121 detected hospitality sector–related clusters, limiting generalizability of our findings.
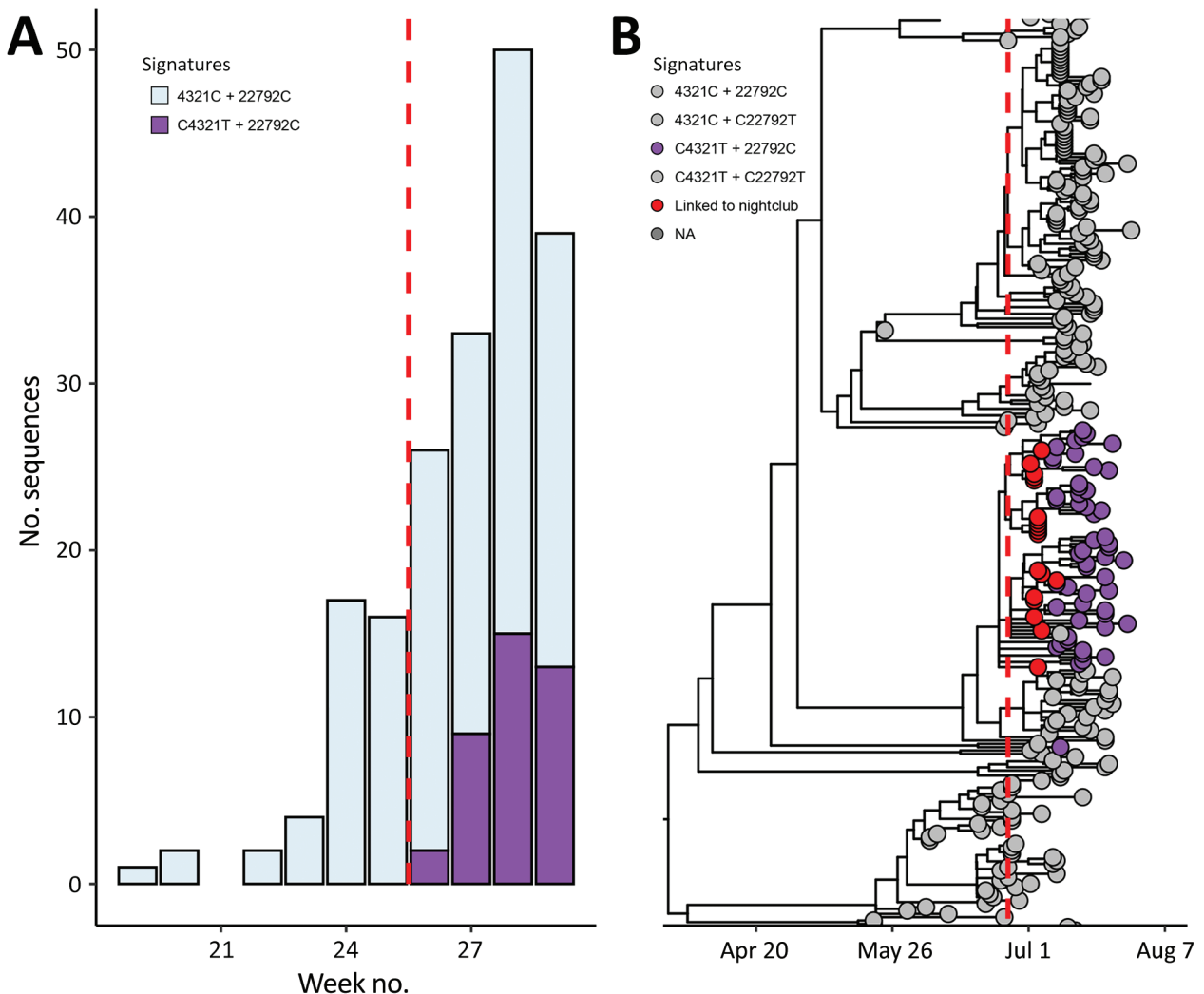
**Figure 2.** Detected increase of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) sequences with signature first detected in nightclub samples, Amsterdam, the Netherlands. A) Absolute number of weekly cases in randomly selected surveillance samples in the Amsterdam region, colored by the nucleotides at position 4321 and 22792 of the SARS-CoV-2 genome. B) Time-resolved phylogenetic tree of dataset containing all Netherlands SARS-CoV-2 Delta variant sequences available in GISAID on August 1, 2021, random surveillance samples from the Amsterdam region, and samples from returning travelers to the Amsterdam region. Tips are colored by the nucleotides at position 4321 and 22792 and epidemiologic linkage to the nightclub (with signature C4321T + 22792C). Dashed red line indicates the day of lifting 1.5-meter social distancing restrictions with QR code. NA, not applicable.

In conclusion, testing-for-access did not prevent superspreading at this event, indicating the need for caution when easing social distancing measures in night life, even under more optimal testing-for-access conditions. This finding is particularly relevant in a population where vaccination coverage is low or when new variants circulate that are associated with lower vaccine effectiveness.

## About the Author

Mr. Koopsen is a PhD candidate at the Amsterdam University Medical Centre. His work focuses on the genomic epidemiology of SARS-CoV-2 and hepatitis C virus. Dr. van Ewijk is a public health physician in training, specializing in infectious disease control. She is also a fellow from the European Programme of Intervention Epidemiology cohort 2021 (European Centre for Disease Prevention and Control) at the National Institute for Public Health and the Environment.

## References

1. Ministry of General Affairs, the Netherlands. The Netherlands takes a big step: almost everything is possible at 1.5 meters [in Dutch]. 2021 Jun 18 [cited 2021 Aug 1]. https://www.rijksoverheid.nl/actueel/nieuws/2021/06/18/nederland-zet-grote-stap-bijna-alles-kan-op-anderhalve-meter
2. Ministry of Health, Welfare and Sport, the Netherlands. CoronaCheck [cited 2021 Aug 1]. https://coronacheck.nl
3. National Institute for Public Health and the Environment, the Netherlands. COVID-19 vaccination program figures [in Dutch]. 2021 [cited 2021 Aug 1]. https://www.rivm.nl/covid-19-vaccinatie/cijfers-vaccinatieprogramma
4. O'Toole Á, Scher E, Underwood A, Jackson B, Hill V, McCrone JT, et al. Assignment of epidemiological lineages in an emerging pandemic using the pangolin tool. Virus Evol. 2021;7:b064. https://doi.org/10.1093/ve/veab064
5. World Health Organization. Tracking SARS-CoV-2 variants. 2021 [cited 2021 Aug 1]. https://www.who.int/en/activities/tracking-SARS-CoV-2-variants
6. Lemieux JE, Siddle KJ, Shaw BM, Loreth C, Schaffner SF, Gladden-Young A, et al. Phylogenetic analysis of SARS-CoV-2 in Boston highlights the impact of superspreading events. Science. 2021;371:eabe3261. https://doi.org/10.1126/science.abe3261
7. Pozderac C, Skinner B. Superspreading of SARS-CoV-2 in the USA. PLoS One. 2021;16:e0248808. https://doi.org/10.1371/journal.pone.0248808
8. Muller N, Kunze M, Steitz F, Saad NJ, Mühlemann B, Beheim-Schwarzbach JI, et al. Severe acute respiratory syndrome coronavirus 2 outbreak related to a nightclub, Germany, 2020. Emerg Infect Dis. 2020;27:645–8. https://doi.org/10.3201/eid2702.204443
9. Kang CR, Lee JY, Park Y, Huh IS, Ham HJ, Han JK, et al.; Seoul Metropolitan Government COVID-19 Rapid Response Team (SCoRR Team). Coronavirus disease exposure and spread from nightclubs, South Korea. Emerg Infect Dis. 2020;26:2499–501. https://doi.org/10.3201/eid2610.202573
10. Greenhalgh T, Jimenez JL, Prather KA, Tufekci Z, Fisman D, Schooley R. Ten scientific reasons in support of airborne transmission of SARS-CoV-2. Lancet. 2021; 397:1603–5. https://doi.org/10.1016/S0140-6736(21)00869-2
11. Centers for Disease Control and Prevention. Scientific brief: SARS-CoV-2 transmission. 2021 May 7 [cited 2022 Feb 10]. https://www.cdc.gov/coronavirus/2019-ncov/science/science-briefs/sars-cov-2-transmission.html
12. Ministry of General Affairs, the Netherlands. Verbatim text press conference Prime Minister Rutte and Minister De Jonge (18 June 2021) [in Dutch] [cited 2021 Aug 1]. https://www.rijksoverheid.nl/documenten/mediateksten/2021/06/18/letterlijke-tekst-persconferentie-minister-president-rutte-en-minister-de-jonge-18-juni-2021
13. Ministry of General Affairs, the Netherlands. Verbatim text press conference Prime Minister Rutte and Minister De Jonge (9 July 2021) [in Dutch] [cited 2021 Aug 1]. https://www.rijksoverheid.nl/documenten/mediateksten/2021/07/09/letterlijke-tekst-persconferentie-minister-president-rutte-en-minister-de-jonge-9-juli-2021
14. National Institute for Public Health and the Environment, the Netherlands. Substantive substantiation of symptomology COVID-19 and consequences for tests and measures [in Dutch] [cited 2021 Aug 1]. https://lci.rivm.nl/onderbouwing-symptomatologie

Address for correspondence: Jelle Koopsen, Amsterdam UMC, Room K3-120, Meibergdreef 9, 1105 AZ, Amsterdam, the Netherlands; email: j.koopsen@amsterdamumc.nl

# Epidemiologic and Genomic Analysis of SARS-CoV-2 Delta Variant Superspreading Event in a Nightclub, the Netherlands, June 2021

## Appendix

## Supplementary Methods

### Virus Detection and Whole-Genome Sequencing

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)–positive materials with cycle threshold values <32 were transported to the Department of Medical Microbiology of the Amsterdam University Medical Centre, location Meibergdreef, for full-genome sequencing. RNA extraction was performed on 200 µL of the original patient sample material by using a MagNaPure 96 System (Roche Diagnostics, https://www.roche.com) according to manufacturer's instructions with a total elution volume of 50 µL. Equine arteritis virus was added before the RNA extraction as an internal extraction control. For cDNA synthesis, the SuperScipt VILO cDNA Synthesis Kit (catalog no. 11754050, ThermoFisher Scientific, https://www.thermofisher.com) was used according to manufacturer's instructions. Extracted RNA was diluted to an estimated input of 100 copies/reaction in nuclease-free water (AM9939, Ambion, ThermoFisher Scientific). In total, 7 µL of diluted RNA solution was combined with 2 µL of 5xVILO reaction mix and 1 µL of 10x SuperScript III enzyme blend tot a total reaction volume of 10 µL. We performed cDNA synthesis on a 96-well Biometra thermal cycler for an initial step at 42°C for 30 minutes followed by 5 minutes at 85°C. We performed SARS-CoV-2 full-genome amplification, adaptor ligation, and purification by using the Ion AmpliSeq SARS-CoV-2 Insight Research Assay (A51305, ThermoFisher Scientific) according to manufacturer's instruction. Libraries were quantified by using the Ion Library TaqMan Quantitation Kit (4468802, ThermoFisher Scientific) according to manufacturer's instructions. Samples were sequenced on an Ion GeneStudio S5 system using an Ion 540 chip (ThermoFisher Scientific). Primer-removed fastq-files were exported for further analysis using the Torrent Suite Software (ThermoFisher Scientific).

Per read quality control was performed using Trimmomatic v0.36 (with settings "LEADING:20 TRAILING:20 SLIDINGWINDOW:10:20 MINLEN:50") (*1*). Resulting quality-checked reads were first mapped to human reference genome HG19 using BWA v0.7.17 (*2*) with default settings ("bwa bwasw") to remove all reads of potential human origin. All unmapped reads were subsequently mapped to SARS-CoV-2 refence genome Wuhan-Hu-1 (*3*). Resulting sequence alignment map (sam) files were converted to bam, sorted, and indexed using SAMtools v1.14 (*4*). A consensus sequence was generated using the TrueConsense package, kindly provided by the National Institute of Public Health and The Environment (RIVM) (sourcecode available at https://github.com/RIVM-bioinformatics/Trueconsense) using settings (-cov 10 -noambig).

**Phylogenetic Analysis**

Surveillance sequences are derived from randomly selected samples from the Amsterdam region to provide an indication of the circulating strains in the region. Returning travelers' surveillance sequences are derived from samples from returning travelers from countries deemed at risk for importation of variants of concern. A maximum-likelihood phylogeny and a time-resolved phylogeny were constructed using the Augur pipeline (*5*). We used procedures taken from [github.com/nextstrain/ncov] including the clock rate, reference genome, site masking, and clade definition files. More specifically, sequences were aligned to reference genome Wuhan-Hu-1 (*3*) using MAFFT (*6*), and a maximum-likelihood tree was constructed using IQ-TREE (*7*) under a HKY+G substitution model, while masking sites 13402, 24389, and 24390. The time-resolved tree was constructed using TreeTime (*8*) using a clock-rate of 0.0008 (SD 0.0004) and after removing molecular clock outliers (tips that deviate >4 interquartile ranges from the root-to-tip vs time regression). Trees were visualized using ggtree (*9*) as implemented in R (*10*). Sequences were deposited in GISAID (EPI_ISL3915592–EPI_ISL_3915610). Pairwise genetic distances were calculated with MEGA6 (*11*) using the distance matrix function with a nucleotide substitution p-distance model. P-distances were transformed to number of single-nucleotide polymorphisms, and are depicted in Appendix Figure 7. One single-nucleotide polymorphism was defined as a p-distance of 0.00003.

**Ethics Statement**

SARS-CoV-2 is a notifiable disease in the Netherlands. The Public Health Service has legal permission, provided by Dutch national public health law, to process patient information for national surveillance of communicable diseases (Wet Publieke Gezondheid,

https://wetten.overheid.nl/BWBR0024705/2021-07-17). Therefore, separate medical ethical clearance for this study was not required.

**References**

1. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30:2114–20. PubMed https://doi.org/10.1093/bioinformatics/btu170

2. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics. 2010;26:589–95. PubMed https://doi.org/10.1093/bioinformatics/btp698

3. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, et al. A new coronavirus associated with human respiratory disease in China [Erratum in: Nature. 2020;580:E7]. Nature. 2020;579:265–9. PubMed https://doi.org/10.1038/s41586-020-2008-3

4. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al.; 1000 Genome Project Data Processing Subgroup. The Sequence Alignment/Map format and SAMtools. Bioinformatics. 2009;25:2078–9. PubMed https://doi.org/10.1093/bioinformatics/btp352

5. Hadfield J, Megill C, Bell SM, Huddleston J, Potter B, Callender C, et al. Nextstrain: real-time tracking of pathogen evolution. Bioinformatics. 2018;34:4121–3. PubMed https://doi.org/10.1093/bioinformatics/bty407

6. Katoh K, Asimenos G, Toh H. Multiple alignment of DNA sequences with MAFFT. Methods Mol Biol. 2009;537:39–64. PubMed https://doi.org/10.1007/978-1-59745-251-9_3

7. Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. 2015;32:268–74. PubMed https://doi.org/10.1093/molbev/msu300

8. Sagulenko P, Puller V, Neher RA. TreeTime: Maximum-likelihood phylodynamic analysis. Virus Evol. 2018;4:vex042. PubMed https://doi.org/10.1093/ve/vex042

9. Yu G. Using ggtree to visualize data on tree-like structures. Curr Protoc Bioinformatics. 2020;69:e96. PubMed https://doi.org/10.1002/cpbi.96

10. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2017.

11. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol. 2013;30:2725–9. PubMed https://doi.org/10.1093/molbev/mst197

12. Ministry of General Affairs, the Netherlands. The Netherlands takes a big step: almost everything is possible at 1.5 meters [in Dutch]. 2021 Jun 18 [cited 2021 Aug 1]. https://www.rijksoverheid.nl/actueel/nieuws/2021/06/18/nederland-zet-grote-stap-bijna-alles-kan-op-anderhalve-meter

13. Ministry of General Affairs, the Netherlands. No choice but to take summertime measures in face of rapid increase in infections [in Dutch]. 2021 Jul 9 [cited 2021 Aug 1]. https://www.government.nl/latest/news/2021/07/09/no-choice-but-to-take-summertime-measures-in-face-of-rapid-increase-in-infections

**Appendix Table.** Sequence characteristics of SARS-CoV-2 infections linked to nightclub event, Amsterdam, the Netherlands, June 2021*

| Sequence | Genome coverage† | Pango lineage | Variant | $C_t$ value | GISAID strain |
|---|---|---|---|---|---|
| AUMC2021AC10 | 99.50% | B.1.617.2 | Delta | 18.90 | hCoV-19/Netherlands/NH-AUMC-004568/2021 |
| AUMC2021AC17 | 99.53% | B.1.617.2 | Delta | 21.58 | hCoV-19/Netherlands/NH-AUMC-004575/2021 |
| AUMC2021AC18 | 99.09% | B.1.617.2 | Delta | 24.23 | hCoV-19/Netherlands/NH-AUMC-004595/2021 |
| AUMC2021AC23 | 99.54% | B.1.617.2 | Delta | 25.72 | hCoV-19/Netherlands/NH-AUMC-004536/2021 |
| AUMC2021AC24 | 99.65% | B.1.617.2 | Delta | 25.17 | hCoV-19/Netherlands/NH-AUMC-004531/2021 |
| AUMC2021AC39 | 0% | NA | NA | 25.57 | Failed sequence |
| AUMC2021AC64 | 99.41% | B.1.617.2 | Delta | 27.98 | hCoV-19/Netherlands/NH-AUMC-004572/2021 |
| AUMC2021AC66 | 99.19% | B.1.617.2 | Delta | 29.81 | hCoV-19/Netherlands/NH-AUMC-004576/2021 |
| AUMC2021AD18 | 98.73% | B.1.617.2 | Delta | 17.16 | hCoV-19/Netherlands/NH-AUMC-004595/2021 |
| AUMC2021AD19 | 99.60% | B.1.617.2 | Delta | 17.81 | hCoV-19/Netherlands/NH-AUMC-004594/2021 |
| AUMC2021AD65 | 97.59% | B.1.617.2 | Delta | 26.22 | hCoV-19/Netherlands/NH-AUMC-004586/2021 |
| AUMC2021AE31 | 99.65% | B.1.617.2 | Delta | 20.32 | hCoV-19/Netherlands/NH-AUMC-009904/2021 |
| AUMC2021AE37 | 99.54% | B.1.617.2 | Delta | 20.08 | hCoV-19/Netherlands/NH-AUMC-009926/2021 |
| AUMC2021AE38 | 99.67% | B.1.617.2 | Delta | 19.44 | hCoV-19/Netherlands/NH-AUMC-009535/2021 |
| AUMC2021AE49 | 99.44% | B.1.617.2 | Delta | 23.07 | hCoV-19/Netherlands/NH-AUMC-009880/2021 |
| AUMC2021AE70 | 99.41% | B.1.617.2 | Delta | 24.70 | hCoV-19/Netherlands/NH-AUMC-009927/2021 |
| AUMC2021AE88 | 99.46% | B.1.617.2 | Delta | 31.48 | hCoV-19/Netherlands/NH-AUMC-009536/2021 |
| AUMC2021AE89 | 99.67% | B.1.617.2 | Delta | 28.75 | hCoV-19/Netherlands/NH-AUMC-009534/2021 |
| AUMC2021AE90 | 99.39% | B.1.617.2 | Delta | 28.01 | hCoV-19/Netherlands/NH-AUMC-009925/2021 |
| AUMC2021AF28 | 99.59% | B.1.617.2 | Delta | 22.70 | hCoV-19/Netherlands/NH-AUMC-009542/2021 |

*$C_t$, cycle threshold; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
†Genome coverage was calculated based on the length of the reference sequence MN908947 (Wuhan-Hu-1) (*3*).
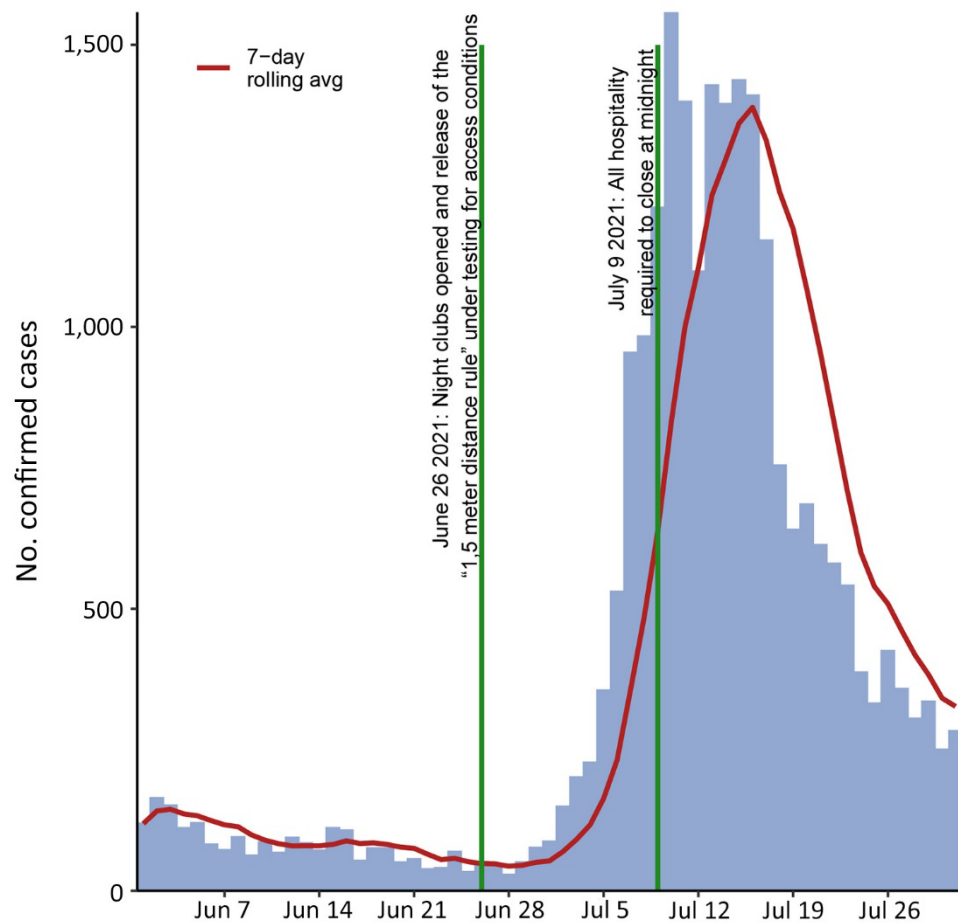
**Appendix Figure 1.** Epicurve of coronavirus disease cases in region of the municipal health service of Amsterdam, the Netherlands. Red line is 7-day rolling average. Green lines indicate dates of measure easing (*12*) and subsequent tightening (*13*).
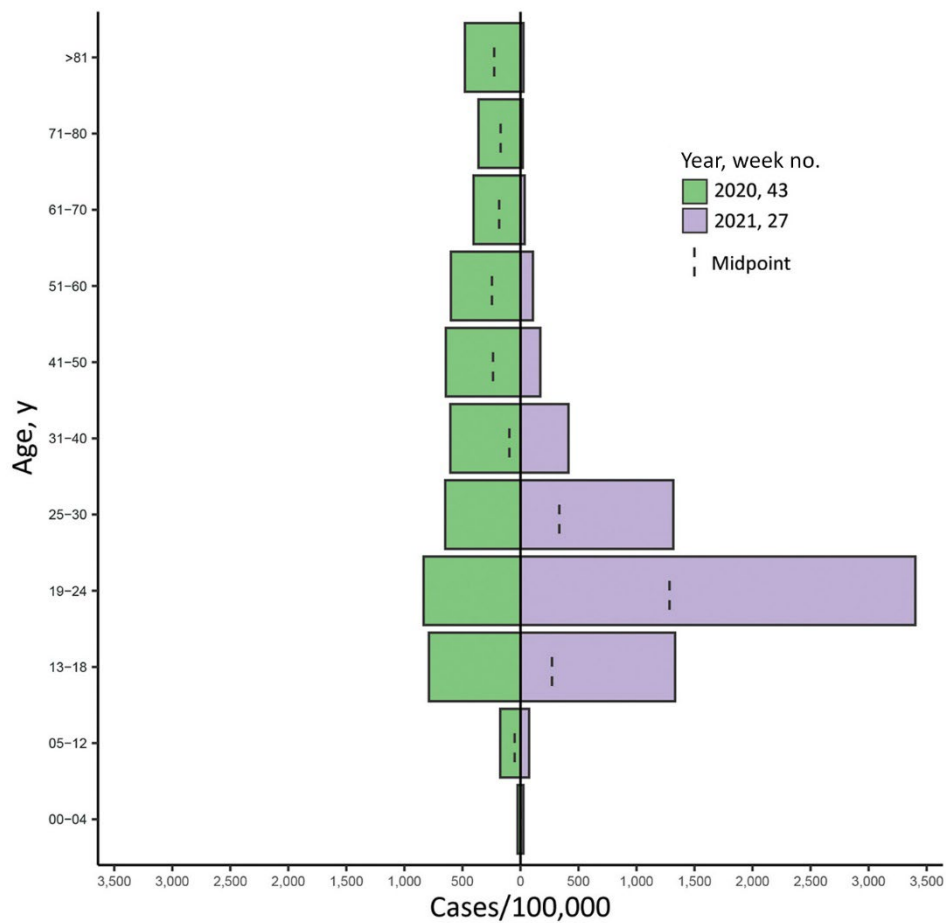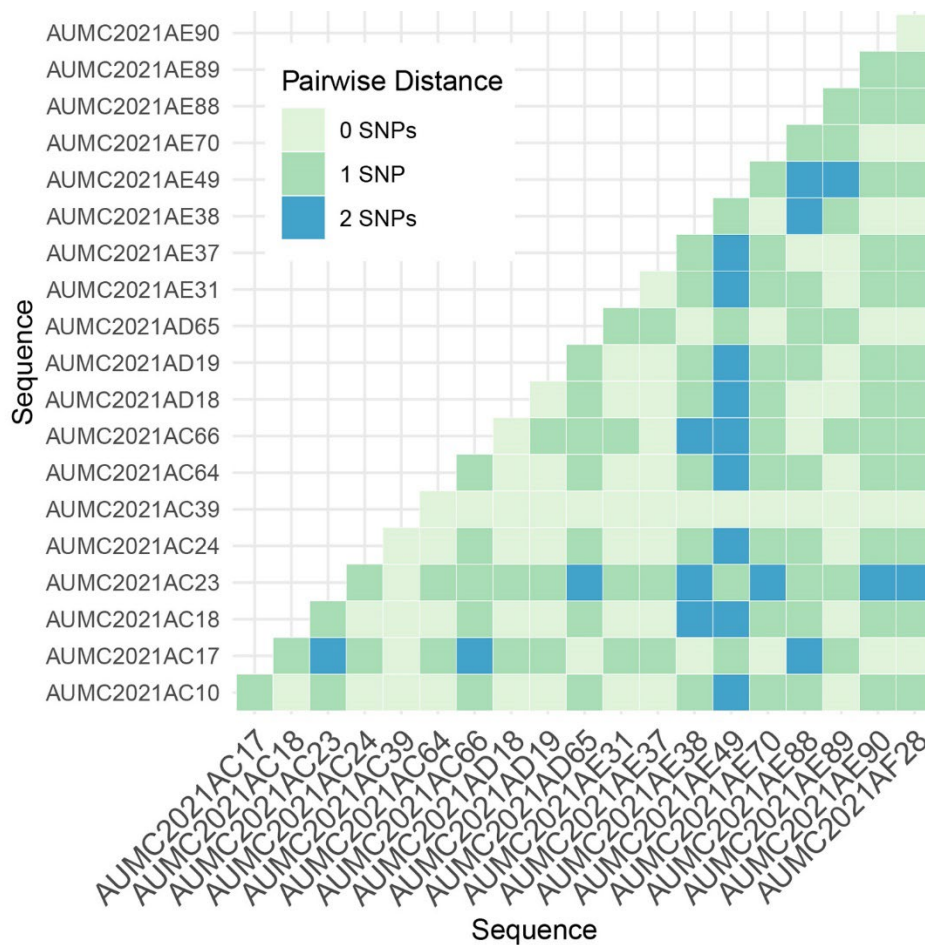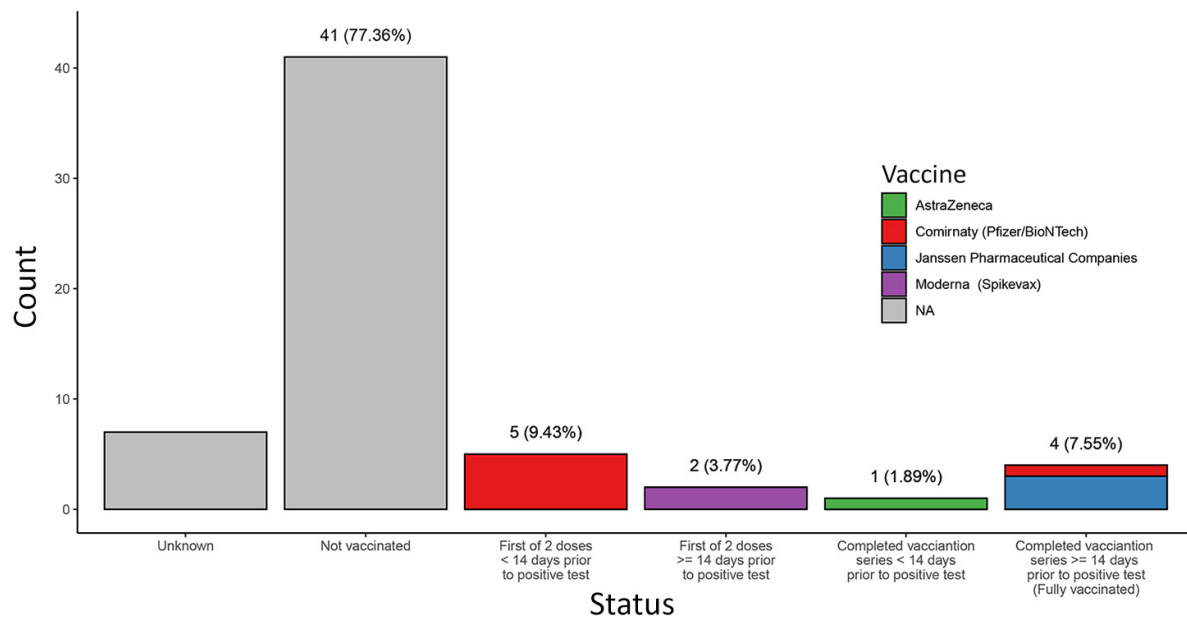
**Appendix Figure 2**. Coronavirus disease cases per 100,000 persons in the Amsterdam region in 2 periods of rapid increase in cases, the Netherlands. Age distributions of all cases in the Amsterdam region in week 43 in 2020 (Autumn 2020 increase) are compared with age distributions of all cases in the Amsterdam region in week 27 in 2021 (most recent increase).
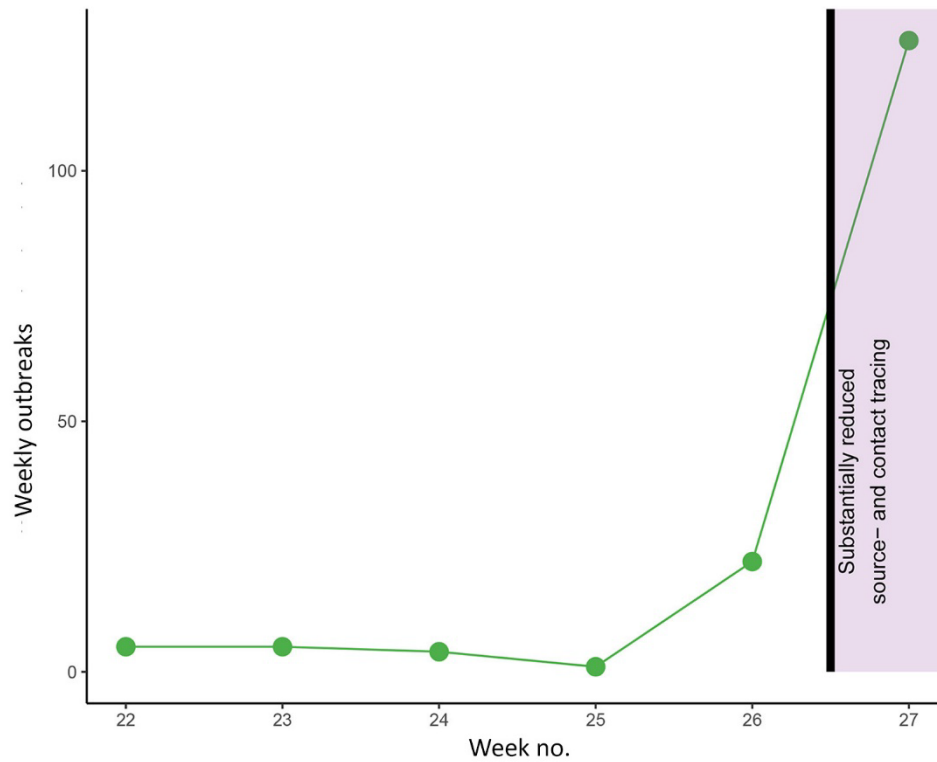
**Appendix Figure 3.** Number of weekly coronavirus disease cases related to hospitality in the Amsterdam region, the Netherlands. Shaded area indicates a period of scaled-down source and contact tracing, indicating underreporting.
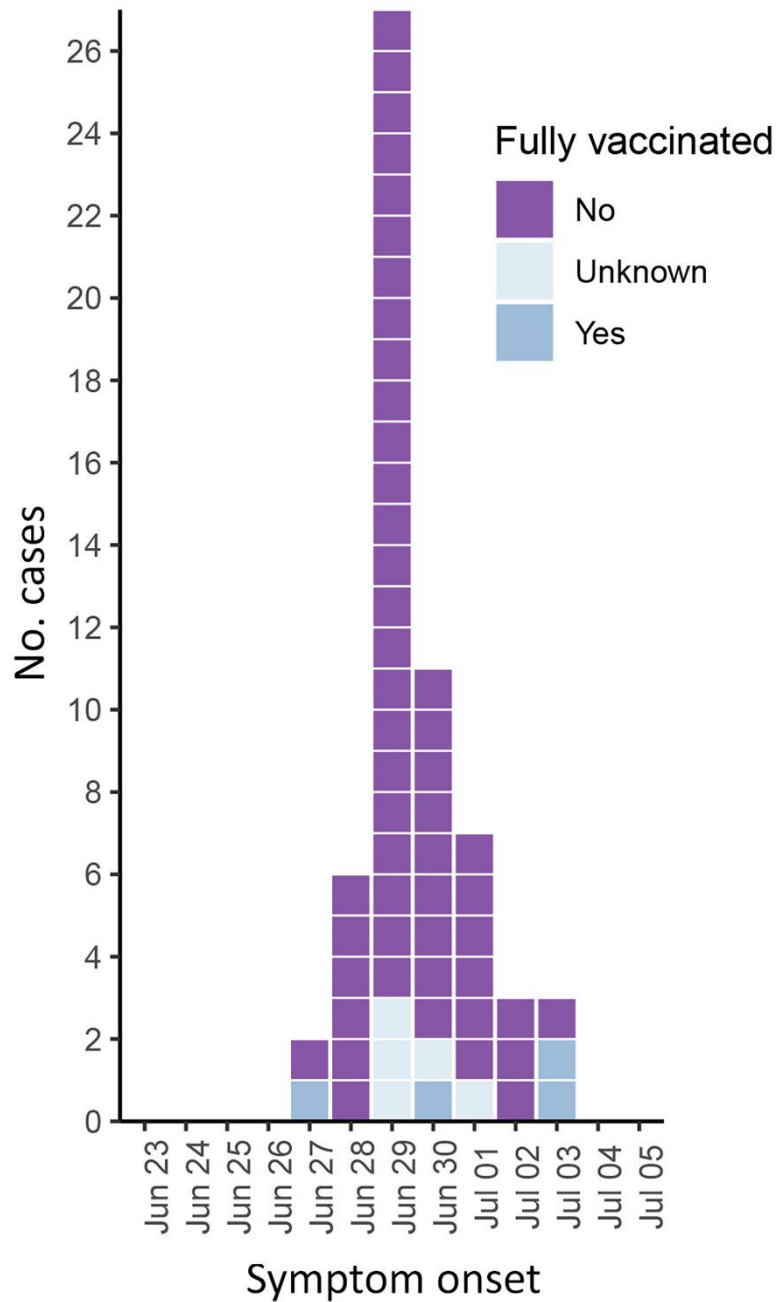
**Appendix Figure 4.** Epicurve of coronavirus disease cases related to a nightclub in central Amsterdam, the Netherlands, on June 26, 2021. Date of symptom onset is depicted, but for asymptomatic cases (n = 4), sampling date of positive test was used. For 1 case, date of symptom onset was missing and date of sampling and was omitted. Fully vaccinated is defined as 14 days after completion of vaccination series.

**Appendix Figure 6.** Pairwise distance matrix of all coronavirus disease sequences linked to event in nightclub, Amsterdam, the Netherlands, June 2021.
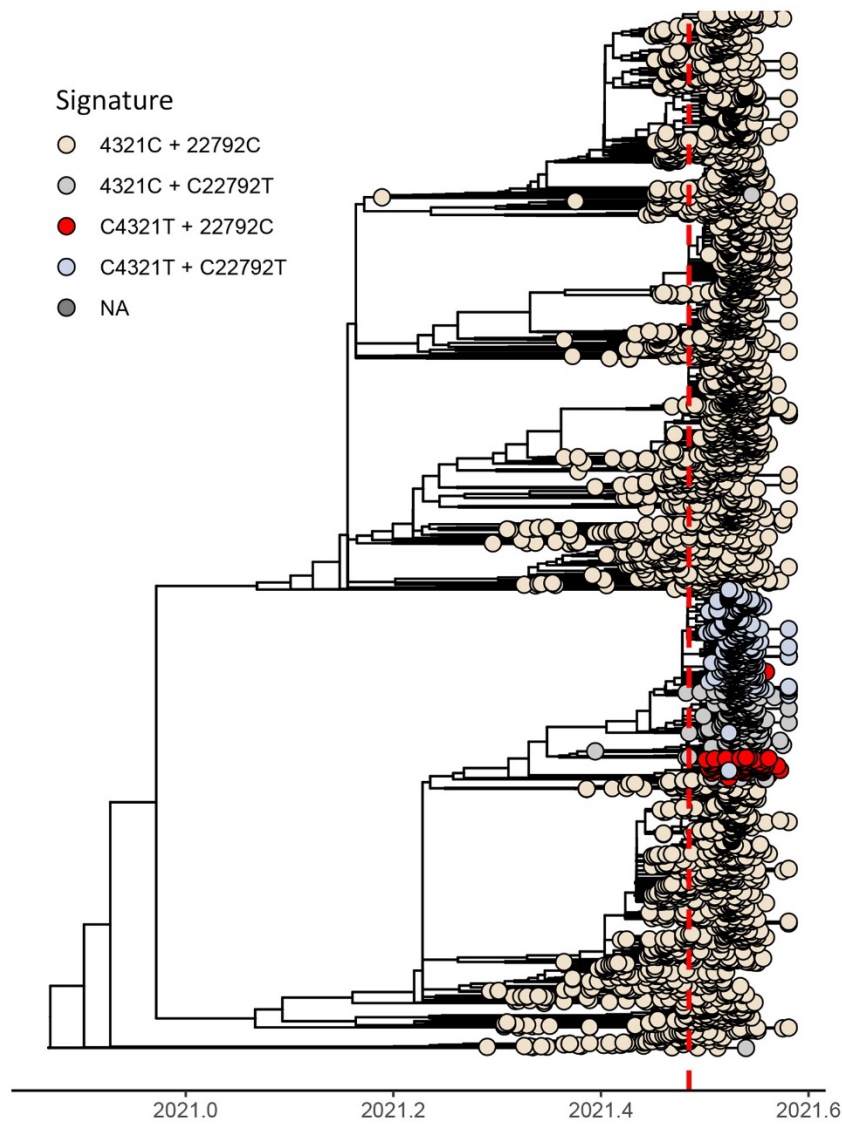
**Appendix Figure 7**. Time-resolved tree of dataset containing all Dutch severe acute respiratory syndrome coronavirus 2 Delta variant sequences available on GISAID on August 1, 2021, random surveillance samples from Amsterdam region, and samples from returning travelers to the Amsterdam region. Red dots denote samples with mutation signature observed in sequences linked to nightclub. Red dashed line indicates June 26, 2021, the date of the event at the nightclub.
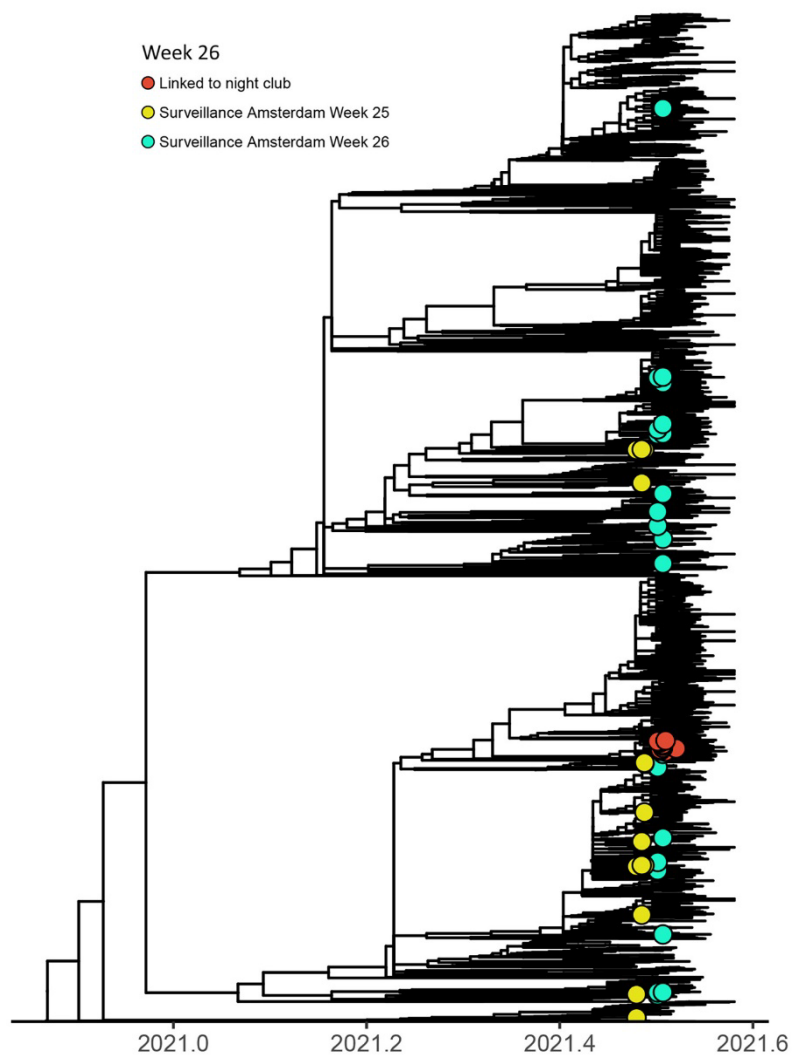
**Appendix Figure 8.** Time-resolved tree of dataset containing all Dutch severe acute respiratory syndrome coronavirus 2 Delta variant sequences available on GISAID on August 1, 2021, random surveillance samples from Amsterdam region, and samples from returning travelers to the Amsterdam region. Random surveillance samples from week 25 and week 26 (2021) are highlighted, together with sequences linked to the nightclub.
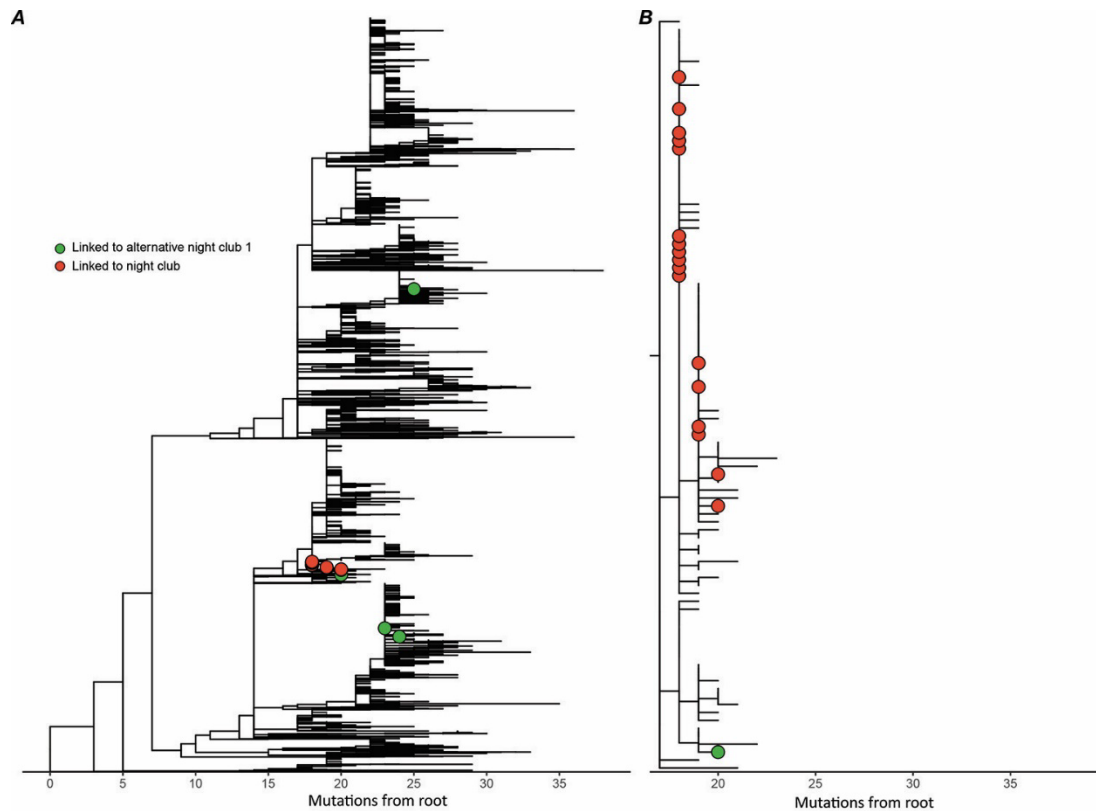
**Appendix Figure 9.** A) Phylogenetic tree containing all Dutch severe acute respiratory syndrome coronavirus 2 Delta variant sequences available on GISAID on August 1, 2021, random surveillance samples from Amsterdam region, samples from returning travelers to the Amsterdam region, and sequences linked to 2 additional nightclub events on June 26, 2021. Sequences linked to alternative nightclub 2 are not shown because they belonged to the B.1.1.7 lineage and were thus unrelated. B) Magnification of the clade containing the nightclub samples.